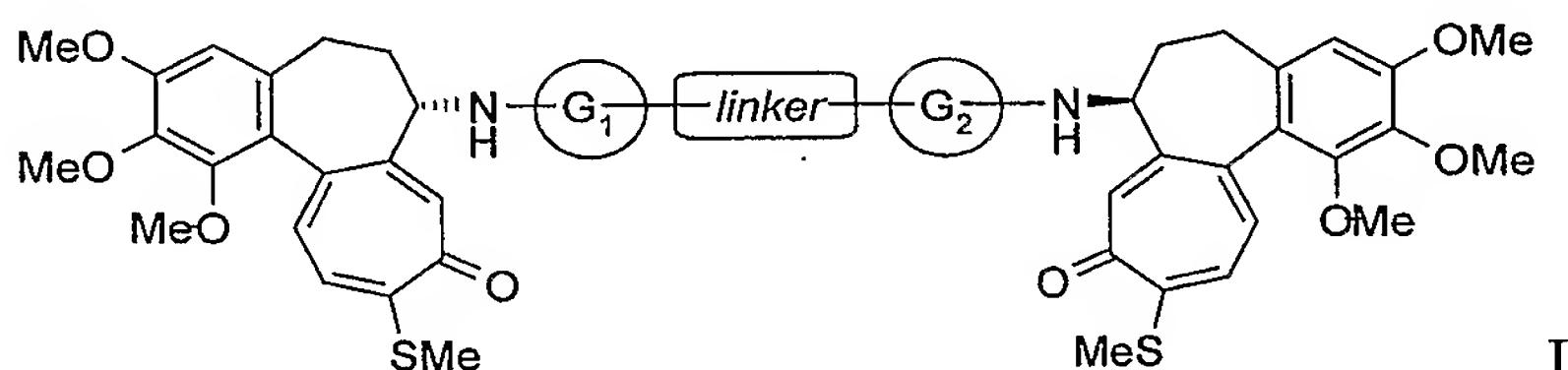


N-DEACETYLTHIOLCOLCHICINE DERIVATIVES, THEIR USE AND PHARMACEUTICAL FORMULATIONS CONTAINING THEM

DISCLOSURE OF THE INVENTION

The present invention relates to N-deacetylthiocolchicine derivatives of formula I



in which:

- the *linker* is a bivalent straight or branched C₁-C₈ alkyl residue, C₃-C₈ cycloalkyl, a phenylene or C₄-C₆ heterocyclic ring;
- the G₁ and G₂, junctions which can be the same or different, are -CO-, -CONH-, -CR₂- groups, in which R₂ is hydrogen or a straight C₁-C₄ alkyl residue,

10 or the G₁-*linker*-G₂ group is the -CO- group.

The compounds of formula I have antiproliferative, antiinflammatory, antiarthritic and antiviral activity.

15 **BACKGROUND OF THE INVENTION**

Colchicine and thiocolchicine have been known for some time in the medical practice. Colchicine is used in the therapy of gout and related inflammatories states. 3-O-Demethylthiocolchicine glucoside is used as miorelaxant in spasticity and muscle pains due to contractures. However, in 20 both cases the use of these compounds is limited due to their high toxicity.

Colchicine and thiocolchicine are also known antiblastic compounds, *ie* compounds that are able to destabilize the microtubules through interaction with tubuline. The possible use of a number of colchicine- and thiocolchicine-

derivatives as antitumor medicaments has been studied. Due to their low therapeutic index none of them has been successful, with the sole exception of demecolchicine, introduced in therapy in the sixties for the treatment of leukemic forms and subsequently replaced by the more effective *Vinca* alkaloids.

WO 01/68597 discloses thiocolchicine dimers in which the thiocolchicine residues are linked through a linear aliphatic amido or amido-ureido bridge.

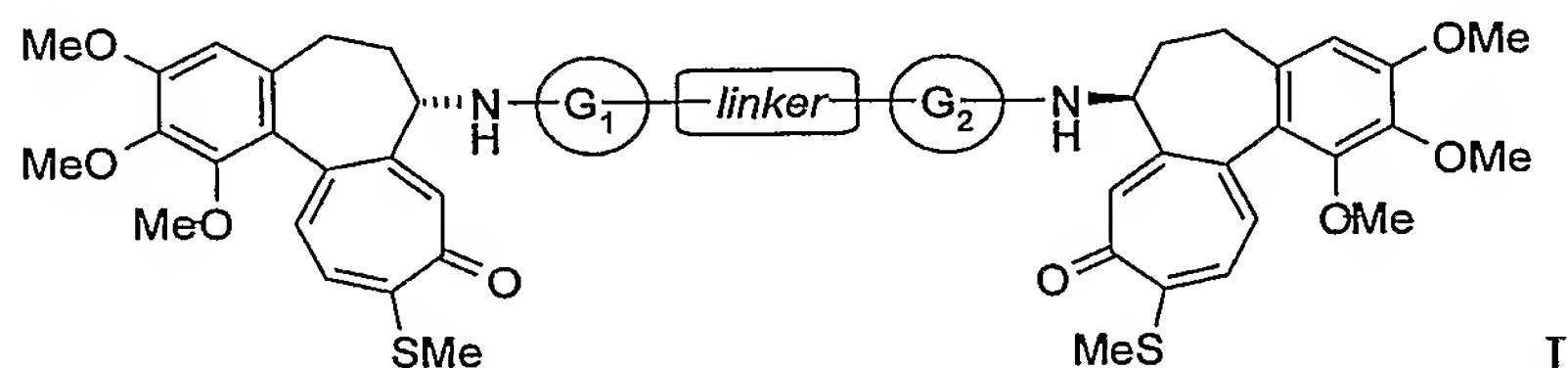
DISCLOSURE OF THE INVENTION

10 It has now been found that the compounds of formula I have antiproliferative activity higher than colchicine and thiocolchicine, in particular on cells expressing the MDR (Multi-Drug Resistance) phenotype.

The compounds of the invention are also more advantageous than the dimers disclosed in WO 01/68597. Moreover, it has been found that the introduction of an aromatic basic residue in the *linker* increases of a dimension order the cytotoxicity of the compounds. The introduction of *linkers* able to properly orient the thiocolchicine residues in the space amplifies the spectrum of activity on resistant tumours. The cytotoxicity of the compounds of formula I proved comparable to that of the most effective antitumour medicaments, whereas the action spectrum is remarkably wider on resistant tumours.

DETAILED DISCLOSURE OF THE INVENTION

Disclosed are N-deacetylthiocolchicine derivatives of formula



25

in which:

- the *linker* is a bivalent straight or branched C₁-C₈ alkyl residue, C₃-C₈ cycloalkyl, a phenylene or a C₄-C₆ heterocyclic ring;
- the G₁ and G₂ junctions, which can be the same or different, are -CO-, -CONH-, -CR₂- groups, wherein R₂ is hydrogen or a straight C₁-C₄ alkyl residue,

5 or the G₁-*linker*-G₂ group is the -CO- group with the proviso that, when G₁ and G₂ are both CO, or when G₁ is -CONH- and G₂ is -CO- the *linker* is different from an alkyl residue.

10 Examples of alkyl bivalent residues comprise straight residues with two, three, four, five or six carbon atoms.

Examples of cycloalkyl groups comprise 1,3-cyclohexylene and 1,4-cyclohexylene.

Examples of phenylene groups comprise 1,2-, 1,3- or 1,4-phenylene.

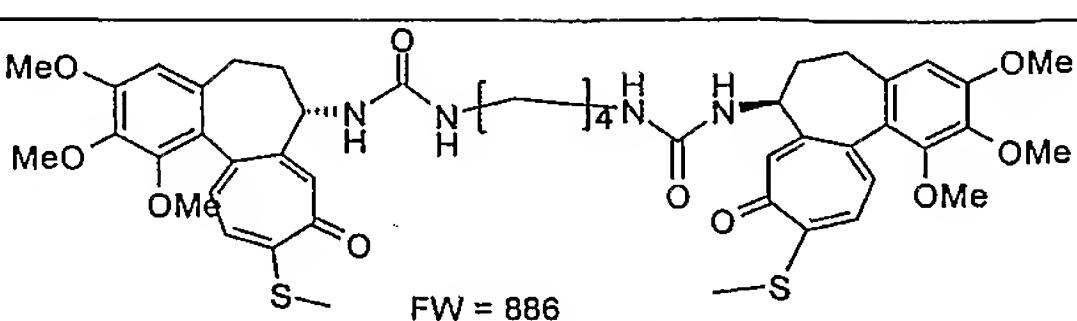
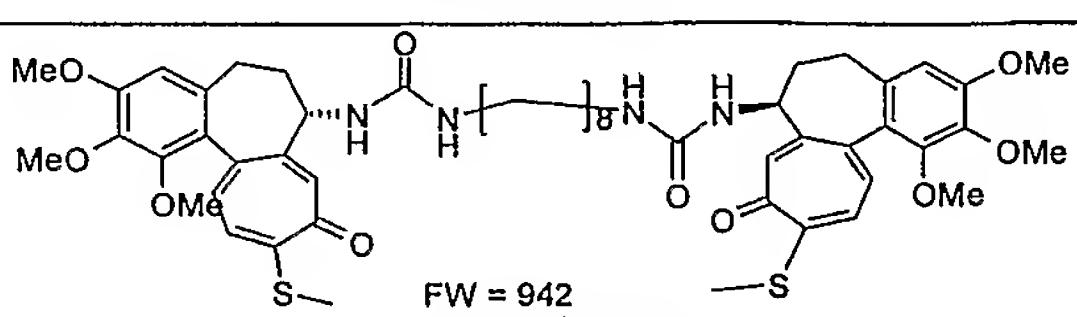
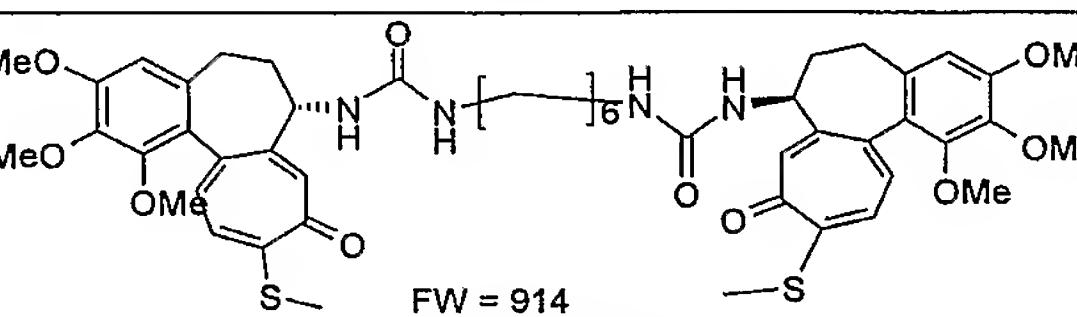
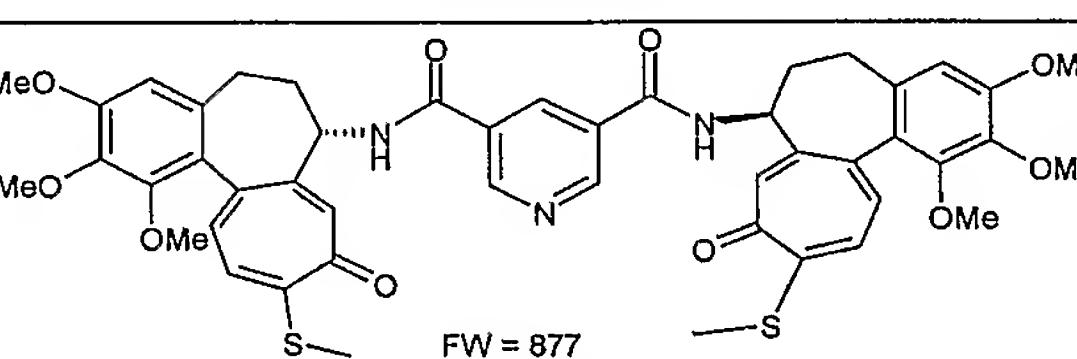
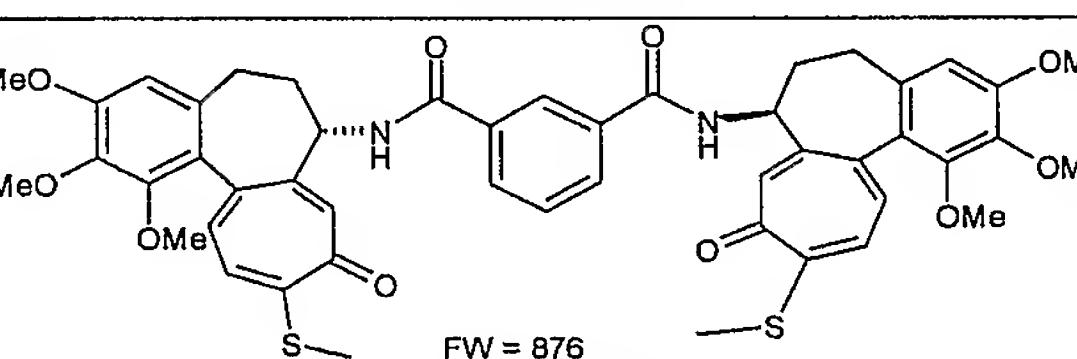
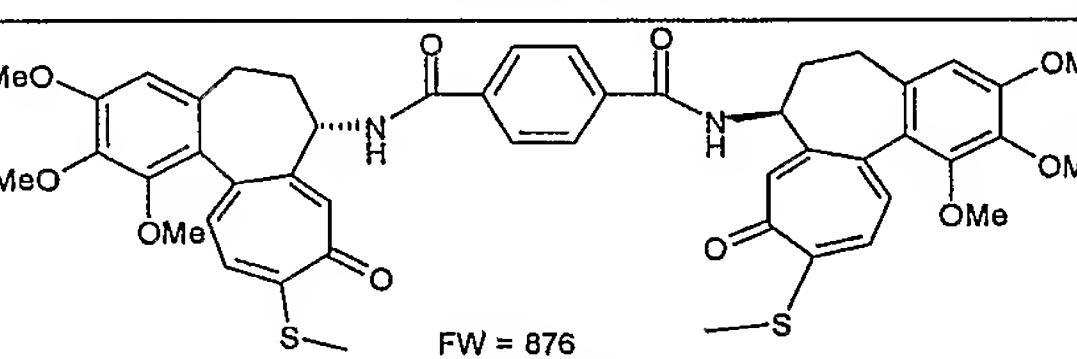
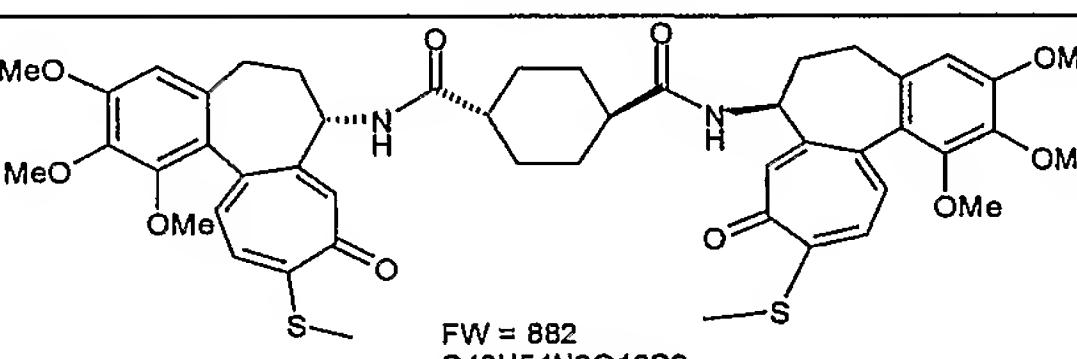
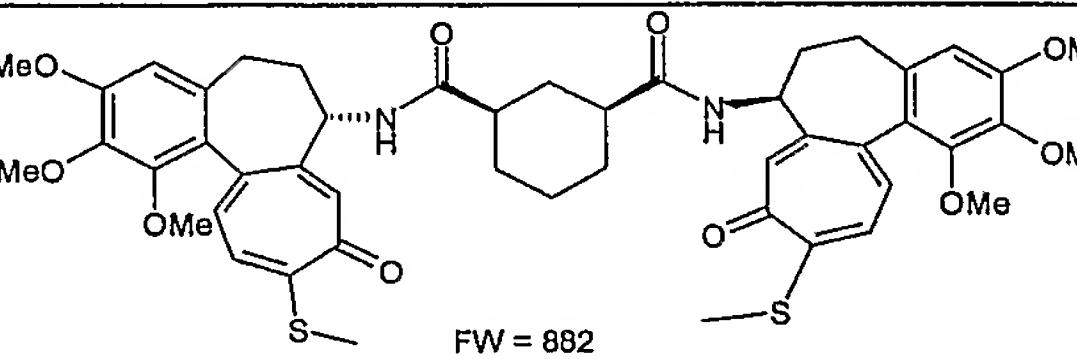
15 Examples of heterocyclic groups comprise pyridyl, pyrazinyl, pyrimidinyl, piperidinyl, piperazinyl linked to the G₁ and G₂ groups through two carbon atoms of the ring, for example in the positions 3,5 or 2,5 or 2,6.

G₁ and G₂ are preferably both CO or CONH.

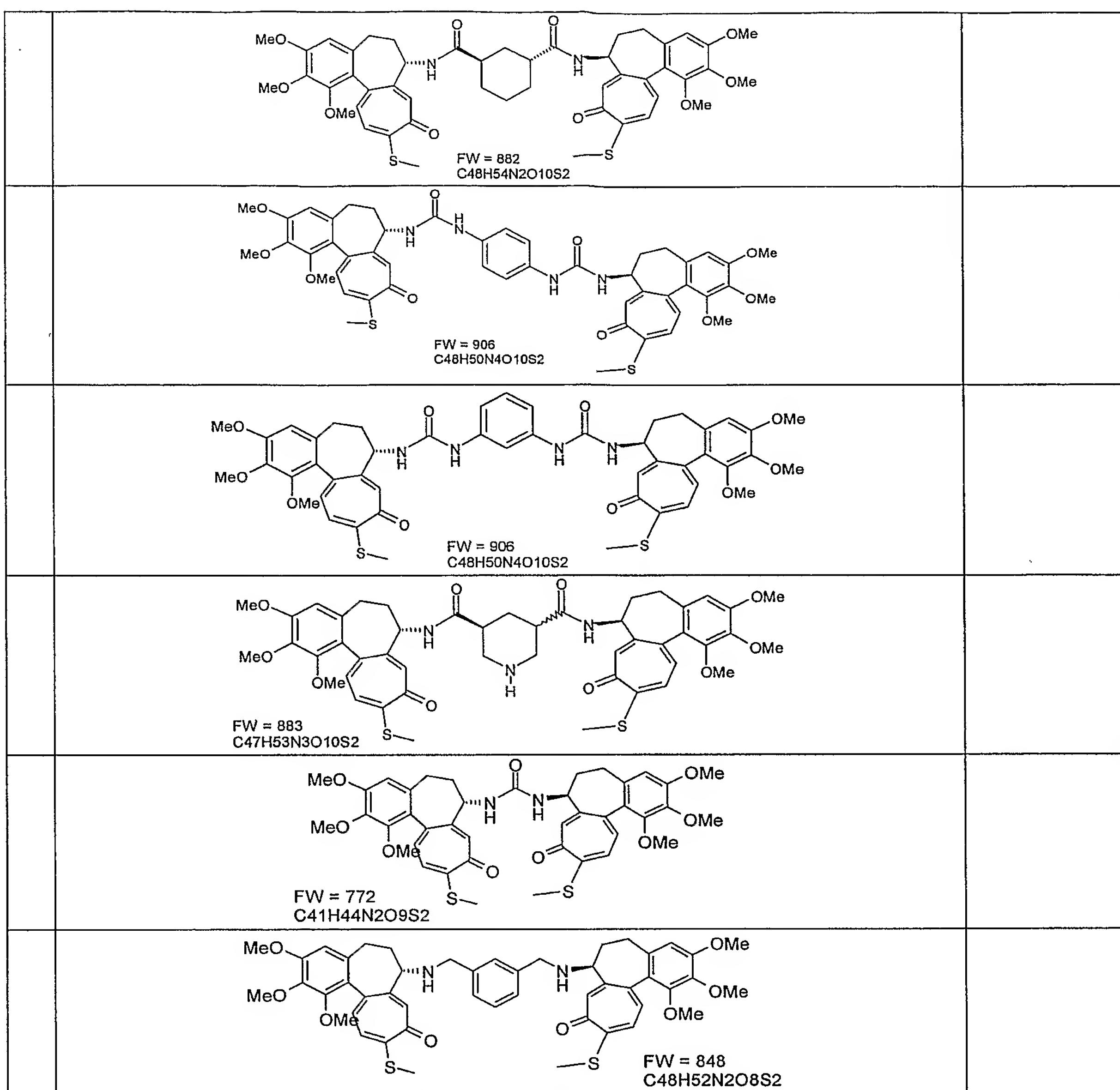
20 The *linker* is preferably a phenylene, cycloalkylene or heterocyclic group as defined above, preferably a heterocyclic group comprising at least one basic nitrogen (pyridyl, pyrimidinyl, pyrazinyl, piperidinyl).

The formulas of some specific compounds of formula I are reported hereinbelow:

Table 1

	 <p>FW = 886 C46H54N4O10S2</p>	
	 <p>FW = 942 C50H62N4O10S2</p>	
	 <p>FW = 914 C48H58N4O10S2</p>	
	 <p>FW = 877 C47H47N3O10S2</p>	
	 <p>FW = 876 C48H48N2O10S2</p>	
	 <p>FW = 876 C48H48N2O10S2</p>	
	 <p>FW = 882 C48H54N2O10S2</p>	
	 <p>FW = 882 C48H54N2O10S2</p>	

Continued



The compounds of formula I have antiproliferative, antinflammatory, antiarthritic and antiviral activity.

The compounds of the present invention are prepared as described in the following.

5 The compounds of the invention of formula I in which $G_1 = G_2 = CO$ (bis-amides) are prepared by reacting N-deacetylthiocolchicine with activated derivatives of the desired dicarboxylic acids in inert solvents. Among activated dicarboxylic acids derivatives, particularly preferred are acid chlorides and mixed anhydrides, particularly with trifluoroacetic acid. Among

inert solvents, particularly preferred are chlorinated solvents. As an alternative, N-deacetylthiocolchicine is reacted with the desired dicarboxylic acid in the presence of DMAP (4-N,N-dimethylaminopyridine) and a condensing agent, DCC (dicyclohexylcarbodiimide), at room temperature, or under heating, and with vigorous stirring until disappearance of the starting products.

The compounds of the invention of formula I in which $G_1 = G_2 = \text{CONH}$ (bis-ureas) are prepared by reacting N-deacetyl-thiocolchicine with the desired bis-isocyanate in an inert solvent. When the selected isocyanate is not commercially available, it is generated in situ by Lossen rearrangement of the corresponding hydroxamic acid by treatment with a carbodiimide and a base. Alternatively, the isocyanate is generated by treatment of the corresponding amide with lead tetraacetate.

The compounds in which G_1 and G_2 are a $-\text{CR}_2-$ group (bis-amines) are prepared by reductive amination of N-deacetylthiocolchicine with suitable dialdehydes. Alternatively, the bis-amines are obtained by alkylation of the thiocolchicine nitrogen with suitable halogen- or tosyl-derivatives. In rare cases the bis-amines are prepared from the corresponding bis-amides through reduction with boranes.

The compounds with different G_1 and G_2 are prepared in two steps through a combination of the methods cited above.

The activity of the compounds of formula I was evaluated on a large number of tumour cells expressing different resistance. The most interesting activity has been observed in ovary, colon, liver and pancreas tumour lines.

Table 2 reports IC_{50} values (expressed in nanomols) of some compounds of the invention in comparison with the dimer disclosed in example 3 of WO 01/68597.

Table 2 - IC_{50}^1 (expressed in nMoles) of thiocolchicine dimers after

72 hours

Compounds	IC ₅₀ (nM) ± S.E.						
	MCF7 (breast)	MCF7-R (MDR+)	A2780 (Ovarian)	A2780/dx (Pgp+)	A2780/top (resistant to topotecan)	A2780/pt (resistant to tocisplatin)	Widr (colon)
E.g. 3 of WO 01/68597	7,9 ± 0,5	33 ± 2,8	238 ± 74	697 ± 192	288 ± 13	252 ± 0,7	351 ± 4
E.g. 1	0,7 ± 0,1	72 ± 4,3	3 ± 0,4	806 ± 286	6,9 ± 4,2	3,7 ± 0,6	25 ± 26,4
E.g. 3	1,5 ± 0,1	108 ± 14	8 ± 5,6	922 ± 164	5,5 ± 0,1	23 ± 4,6	48 ± 24
E.g. 2	14 ± 0,9	135 ± 11	37 ± 5	1273 ± 382	32 ± 24	26 ± 1,0	230 ± 166
E.g. 4			252 ± 38	3841 ± 224	152 ± 36	331 ± 63	650 ± 40

¹ IC₅₀: concentration that inhibits cell growth by 50% compared with non treated cells.

5 Cytotoxic activity was evaluated according to the procedure described by M.C. Alley et al., *Cancer Research* 1998, 48, 589-601.

All the thiocolchicine dimers with the claimed spacers proved to possess antiproliferative, antiinflammatory and antiviral activity. These compounds are therefore useful in the treatment of neoplasias of various 10 origin, deforming rheumatoid arthritis and in the treatment of the Kaposi tumour whose retroviral component is ascertained. The inhibiting action of viral replication combined with the inhibition of cell proliferation in actively proliferating tissues is of particular interest, considering the origin of several human tumours.

15 For this purpose, the compounds of the invention will be administered as pharmaceutical compositions suitable for intravenous, parenteral, oral, transdermal administration. A particularly useful aspect of the invention relates to the preparation of complexes with plasma proteins, in particular

engineered human albumin. The protein complexes of these derivatives are obtained adding to a concentrated solution of albumin the compound dissolved in dioxane in a time that allows the reaction between the involved molecular species. After reacting the two molecular species (albumin and the colchicine derivative) under physiological pH and ionic strength conditions, the resulting solution is lyophilised. If the lyophilised solution is prepared under conditions of absolute sterility, it is ready for endovenous injection; the lyophilised solution, after dispersion in suitable and compatible excipients, can be compressed and administered through the oral route providing plasma concentrations of medicaments close to those obtainable though the parenteral route. As an alternative to the use of plasma proteins, given the poor water solubility of the compounds, variously functionalised cyclodextrins or acryl matrices suitable for parenteral administration in humans can be advantageously used.

Depending on the administration route, the dosage of the compounds will range from 1 to 20 mg/m² body area. The preferred administration routes are locoregional injection, intravenous and oral administration.

Examples of compositions comprise freeze-dried vials, supported lyophilised tablets and drinkable solutions. Any other agents that dissolve the compounds with to an acceptable extent for administration, such as Tween, Cremophor and suitable mixtures thereof with PEG or alcohols are also comprised in the present invention.

The following examples illustrate the invention in greater detail.

EXAMPLES

Example 1 - 3,5-Pyridinedicarboxylic acid bis-(N-deacetylthiocolchicine) amide

10 g of N-deacetylthiocolchicine are dissolved in 60 ml of methylene chloride. 2.24 g (0.5 eq) of 3,5-pyridinecarboxylic acid, 1.64 g (0.5 eq) of

N,N-dimethylaminopyridine (DMAP) and 8.3 g (1.5 eq) of dicyclohexylcarbodiimide (DCC) are then added under vigorous stirring. The mixture is left under stirring, monitoring the reaction by TLC (AcOEt:MeOH 10:1), until disappearance of the reagents (about 12 hours). The solution is 5 then filtered through Celite, washing the pad with methylene chloride (2x100 ml). The combined organic phases are washed first with an equal volume of 1N HCl and then with 1N NaOH. The organic phase is dried over sodium sulfate and evaporated under vacuum. The residue is purified by filtration on silica gel (AcOEt:MeOH 10:1). The resulting product is dried 10 overnight in a static dryer at 40°C under vacuum to give 7.5 g of final product.

¹HNMR (300 MHz, CDCl₃): 1.00-1.42 (m, 2H), 1.50-2.00 (m, 2H), 2.05-2.30 (m, 2H), 2.30-2.50 (m, 2H), 2.52 (s, 3H, SMe), 3.71 (s, 3H, MeO-1), 3.90 (s, 3H, MeO-2), 3.96 (s, 3H, MeO-3), 4.80-4.92 (m, 2H, H-7), 6.47 (s, 2H, H-8), 7.18 (d, 12.0 Hz, 2H, H-12), 7.38 (d, 12.0 Hz, 2H, H-11), 7.67 (s, 2H, H-4), 8.52 (br s, 2H, NH), 9.28 (s, 2H, H-2'+H-6'); 9.60 (s, 1H, H-4');

¹³CNMR (75 MHz, CDCl₃): 182.64, 164.25, 158.88, 154.12, 152.59, 152.34, 151.33, 141.92, 139.33, 135.48, 134.47, 132.74, 129.04, 128.54, 127.48, 125.71, 107.74, 61.88, 61.61, 56.49, 53.03, 30.09, 15.41.

IR (KBr): 2934, 2854, 1664, 1605, 1540, 1485, 1424, 1403, 1349, 1321, 20 1266, 1235, 1195, 1153, 1137, 1095, 1051, 1021, 978, 921, 842, 796, 703.

Example 2 - *trans*-1,4-Cyclohexanedicarboxylic acid bis-(N-deacetylthiocolchicine) amide

10 g of N-deacetylthiocolchicine are dissolved in 60 ml of methylene chloride. 2.31 g (0.5 eq) of *trans*-1,4-cyclohexanedicarboxylic acid, 1.64 g 25 (0.5 eq) of DMAP and 8.3 g (1.5 eq) of DCC are then added under vigorous stirring. The mixture is left under stirring for 12 hours and monitored by TLC (AcOEt:MeOH 10:1), then filtered through Celite, washing the pad with methylene chloride (2x20 ml). The combined organic phases are washed with

an equal volume of 1N HCl, 1N NaOH and brine, dried over Na_2SO_4 . After evaporation of the solvent under reduced pressure, the residue is purified by direct column chromatography (AcOEt:MeOH 12:1). The product is crystallized from methanol (10 v/v) and dried overnight in a static dryer at 5 40°C under vacuum to give 6.6 g of pure compound.

^1H NMR (300 MHz, CDCl_3): 1.30-1.45 (m), 1.45-1.70 (m, 2H), 1.80-1.89 (m), 1.89-2.10 (m), 2.18-2.56 (m), 2.43 (s, 3H, SMe), 3.69 (s, 3H, MeO-1), 3.92 (s, 3H, MeO-2), 3.97 (s, 3H, MeO-3), 4.80-4.92 (m, 2H, H-7), 6.47 (s, 2H, H-8), 7.09 (d, 12.0 Hz, 2H, H-12), 7.34 (d, 12.0 Hz, 2H, H-11), 10 7.86 (s, 2H, H-4), 8.93 (br s, 2H, NH).

^{13}C NMR (75 MHz, CDCl_3): 182.44, 176.35, 158.52, 153.80, 152.96, 151.55, 141.97, 139.22, 135.04, 134.69, 129.93, 127.09, 126.37, 107.04, 62.39, 61.68, 56.45, 51.13, 44.59, 36.92, 30.63, 29.61, 27.84, 15.36.

IR (KBr): 3442, 3285, 2934, 2855, 1674, 1602, 1532, 1484, 1454, 1424, 15 1403, 1390, 1348, 1321, 1281, 1256, 1236, 1195, 1153, 1136, 1094, 1022, 982, 942, 921, 841, 619, 582.

Example 3 - 3,5-Benzenedicarboxylic acid bis-(N-deacetylthiocolchicine) amide

The procedure of example 1 is followed starting from 5 g of isophthalic 20 acid to obtain the product as a crystalline solid (yield: 82%).

^1H NMR (300 MHz, CDCl_3): 1.00-1.42 (m, 2H), 1.50-2.00 (m, 2H), 2.05-2.30 (m, 2H), 2.30-2.50 (m, 2H), 2.52 (s, 3H, SMe), 3.71 (s, 3H, MeO-1), 3.90 (s, 3H, MeO-2), 3.96 (s, 3H, MeO-3), 4.80-4.92 (m, 2H, H-7), 6.47 (s, 2H, H-8), 7.18 (d, 12.0 Hz, 2H, H-12), 7.38 (d, 12.0 Hz, 2H, H-11), 7.67 25 (s, 2H, H-4), 8.52 (br s, 2H, NH), 8.05 (m, 2H, H-4'+H-6'), 8.50 (m, 1H, H-2'), 7.46 (dd, 8.0 Hz, H-5').

Example 4 - N-Deacetylthiocolchicine 1,4-phenylenediamine bis-urea

2.0 g of N-deacetylthiocolchicine are dissolved in 150 ml of dry

tetrahydrofuran. 0.5 equivalents of 1,4-phenylene diisocyanate (0.4 g) are then added. The reaction mixture is left under stirring at room temperature for two days, monitoring by TLC (DCM-EtOH 95:5, $R_f=0.30$). The solvent is evaporated off and the crude is recrystallized from ethyl acetate to obtain 5 0.95 g of pure product (38%).

^1H NMR (300 MHz, DMSO-d6): 1.72-1.88 (m), 2.08-2.36 (m), 2.58-2.70 (m), 2.42 (s, 3H, SMe), 3.58 (s, 3H, MeO-1), 3.82 (s, 3H, MeO-2), 3.87 (s, 3H, MeO-3), 4.24-4.35 (m, 2H, H-7), 6.82 (s, 2H, H-8), 7.17 (d, 12.0 Hz, 2H, H-12), 7.27 (d, 12.0 Hz, 2H, H-11), 7.13 (s, 2H, H-4), 8.52 (br s, 2H, 10 NH).

Example 5 - n-Deacetylthiocolchicine 1,3-phenylenediamine bis-urea

The procedure of example IV is followed starting from 2.0 g of N-deacetylthiocolchicine and 0.4 g of 1,3-phenylene diisocyanate to obtain the 15 desired product (yield: 42%).

^1H NMR (300 MHz, DMSO-d6): 1.72-1.88 (m), 2.08-2.36 (m), 2.58-2.68 (m), 2.43 (s, 3H, SMe), 3.58 (s, 3H, MeO-1), 3.83 (s, 3H, MeO-2), 3.87 (s, 3H, MeO-3), 4.24-4.35 (m, 2H, H-7), 6.82 (s, 2H, H-8), 7.18 (d, 12.0 Hz, 2H, H-12), 7.28 (d, 12.0 Hz, 2H, H-11), 7.14 (s, 2H, H-4), 8.48 (br s, 2H, 20 NH).

Example 6 - 2,9-Diazasebacic acid bis-(N-deacetylthiocolchicine) amide

The procedure of example IV is followed starting from 2.0 g of N-deacetylthiocolchicine and 0.4 g of 1,4-butanediisocyanate to obtain the 25 desired product (yield: 63%, 0.4 g). TLC (DCM-EtOH=95:5) $R_f=0.38$.

^1H NMR (300 MHz, CDCl_3): 1.80-1.95 (m), 2.38-2.54 (m), 2.70-2.95 (m), 3.40-3.60 (m), 2.50 (s, 3H, SMe), 3.69 (s, 3H, MeO-1), 3.95 (s, 3H, MeO-2), 3.98 (s, 3H, MeO-3), 4.54-4.65 (m, 2H, H-7), 6.58 (s, 2H, H-8), 7.21

(d, 12.0 Hz, 2H, H-12), 7.43 (d, 12.0 Hz, 2H, H-11), 7.81 (s, 2H, H-4), 8.20 (br s, 2H, NH).

Example 7 - 1,3-Benzenemethylene-bis-N-deacetylthiocolchicine

2.0 g of N-deacetylthiocolchicine are dissolved in 100 ml of chloroform. 0,5 Equivalents of isophtalic aldehyde dimethylacetal and 0.01% of pyridinium tosylate are added. The mixture is refluxed overnight, allowed to cool down to room temperature and placed in an ice bath. 8 Equivalents of sodium triacetoxy borohydride are added and the mixture is left under stirring for 1 day. The solution is then filtered, washed with the same volume of 0.1 N HCl and then with saturated aqueous sodium bicarbonate. The organic phase is dried over sodium sulfate and the solvent is evaporated off. The crude is purified by flash chromatography to give 0,49 g of product.

¹HNMR (300 MHz, CDCl₃): 1.00-1.42 (m, 2H), 1.50-2.00 (m, 2H), 2.05-2.30 (m, 2H), 2.30-2.50 (m, 2H), 2.52 (s, 3H, SMe), 3.71 (s, 3H, MeO-1), 15 3.90 (s, 3H, MeO-2), 3.96 (s, 3H, MeO-3), 4.80-4.92 (m, 2H, H-7), 6.47 (s, 2H, H-8), 7.18 (d, 12.0 Hz, 2H, H-12), 7.38 (d, 12.0 Hz, 2H, H-11), 7.67 (s, 2H, H-4), 8.52 (br s, 2H, NH), 7.02 (m, 5H).

Example 8 - Injectable formulation of 3,5-pyridinedicarboxylic acid bis-(N-deacetylthiocolchicine) amide complexed with human albumin

20 1 g of 3,5-pyridinedicarboxylic acid bis-(N-deacetylthiocolchicine) amide is dissolved in 20 ml of dioxane. The resulting solution is slowly dropped into a 5% physiological solution of albumin to obtain a homogeneous milky suspension. The mixture is left under stirring in sterile conditions for two hours, then lyophilised.

25 The lyophilised product is ready for administration through injection route.